On the total synthesis of terpenes containing quaternary stereocenters

Buter, Jeffrey

English Summary

On the total synthesis of terpenes containing quaternary stereocenters; Stereoselective synthesis of the taiwaniaquinoids, mastigophorene A, and tuberculosinyl adenosine

The total synthesis of naturally occurring compounds is a field of research with a long-standing tradition and importance. As a matter of fact, the first synthesis of a natural product, that of urea by Friedrich Wöhler in 1828, spawned the field of organic chemistry. Over the past 200 years an enormous amount of natural products have been synthesized and these had a decisive impact on fields like biochemistry, medicine and biology. Examples of how natural product synthesis benefits daily life are: *the industrial production of vitamins and amino acids, the use of volatile compounds in perfumes and fragrances, and natural products that serve as leads or are actual medicines.*

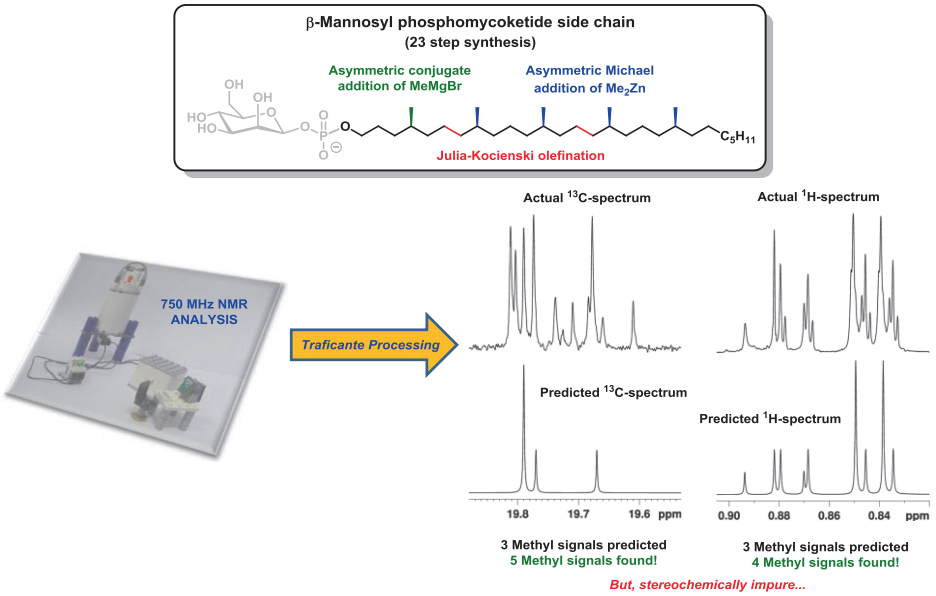
From an academic perspective the practice of organic chemistry is largely driven by curiosity, the development of novel methodology, and the discovery of new reactivity to construct molecules. Natural product synthesis in particular serves as an ideal platform to scrutinize new synthesis methodology by testing it in a complex setting (*complex chemical structures*). The synthesis of a complex natural product elicits the development of new methodology as synthesis in such a complex setting often requires modification of reaction conditions.

In this dissertation the reader will find a detailed description of my work in the field of natural product synthesis. The research focused on the stereoselective synthesis of naturally occurring compounds exhibiting a quaternary stereocenter, a chemical motive generally difficult to construct. In this thesis one will find the development of novel methodology and applications of new chemical reactions within the confines of natural product synthesis. Additionally, our synthetic efforts assisted in the structure elucidation of a novel terpene nucleoside from *Mycobacterium tuberculosis*, confirming its biosynthesis, and producing quantities of material for further biochemical and immunological studies. We also used a natural product, mycoketide, to develop an NMR-based approach to establish the stereochemistry of oligoisoprenoids.

Chapter 2 describes the asymmetric synthesis of this β-mannosyl phosphomycoketide side chain and subsequent NMR analysis thereof (Scheme 1). Exhibiting an all-*syn* 1,5-methyl ramification with five repeating stereogenic methine groups , the determination of the relative stereochemistry and isomeric purity by means of NMR analysis is particularly challenging.

In 2013, Curran and co-workers synthesized all possible (relative) stereoisomers of shorter analogues of the mycoketide side chain containing three repeating methyl branches. It was found that the ^{1}H- and ^{13}C-NMR spectra (750 MHz) were very similar but not identical. Assignment of the specific resonances, for the determination of the isomeric purity, proved to be impossible however as the signals were in too close proximity. Processing of the acquired data with the Traficante algorithm (resolution

enhancement) separated the overlapping signals which, in combination with computational simulation of the spectra, allowed the assignment of the resonances and therefore assessment of the diastereomeric ratio.



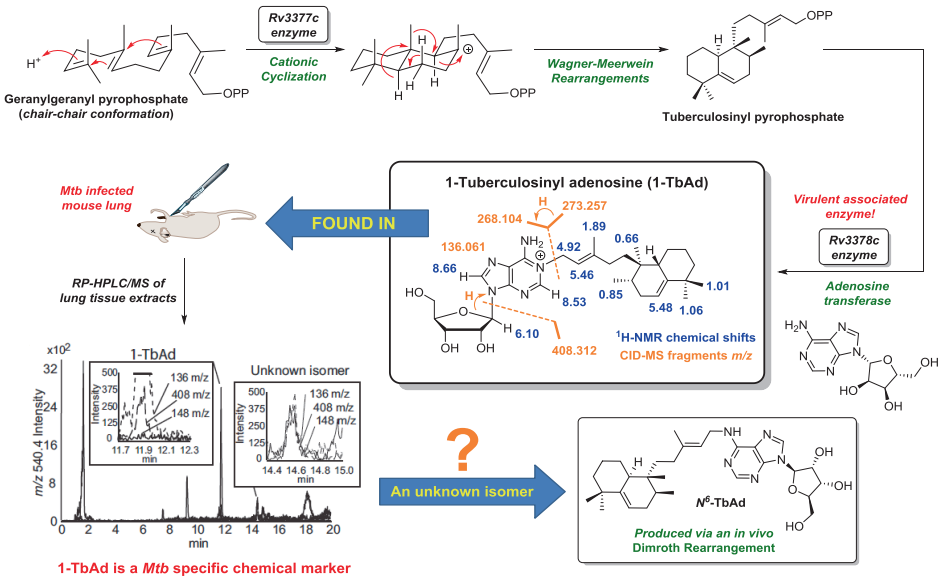
Scheme 1. *Synthesis and NMR analysis of all-(S) mycoketide.*

Based on the obtained empirical data the Curran laboratory was able to produce a set of predicted spectra for higher oligoisoprenoids like the phosphomycoketide side chain. To test the validity of this model, actual material was necessary. This material was provided to the Curran laboratory in the form of all-*syn* mycoketide, and a smaller four methyl groups containing analogue.
NMR analysis of the synthetic material showed that somewhere in the synthesis erosion of enantiopurity had taken place, leading to ~70% isomeric purity of the product. However, the obtained ^{13}C-NMR spectrum, after Traficante processing, showed five separate resonances for the five methyl groups, whereas the predicted spectra were unable to distinguish the three central methyl groups. This result was surprising as the chemical environment of the three central methyl groups is very similar. In the ^{1}H-NMR spectrum of mycoketide, four of the methyl groups were visible. The research allowed therefore expansion of the model for the prediction of the relative stereochemistry and/or stereopurity assessment of saturated oligoisoprenoids, a common motive in natural products.

The third chapter of this dissertation is the result of a collaboration of several research groups, coordinated by the Moody laboratory at Harvard Medical School. In 2012, Moody and co-workers isolated an unknown, but abundant, natural product from the pathogen *Mycobacterium tuberculosis*. As *Mycobacterium tuberculosis* is responsible

for over 1.5 million deaths annually, the identification of novel molecules is important as this provides insight in the, survival and virulence mechanism of the bacterium. Additionally, pathogen-specific compounds might find application as chemical markers for diagnostic tests for the tuberculosis disease.

Investigation into the molecular architecture of the unknown isolate using mass spectrometry and NMR analysis, led to its assignment as 1-tuberculosinyl adenosine (1-TbAd) (Scheme 2). A total synthesis of the racemate confirmed its structure, and also provided material for initial investigations. As a part of the chemical synthesis, its putative biosynthetic precursor tuberculosinyl pyrophosphate was also produced. With this material the biosynthesis of 1-TbAd was shown to involve the virulence associated enzyme Rv3378c, indicating that this molecule might be important for inducing virulence of *Mycobacterium tuberculosis*.



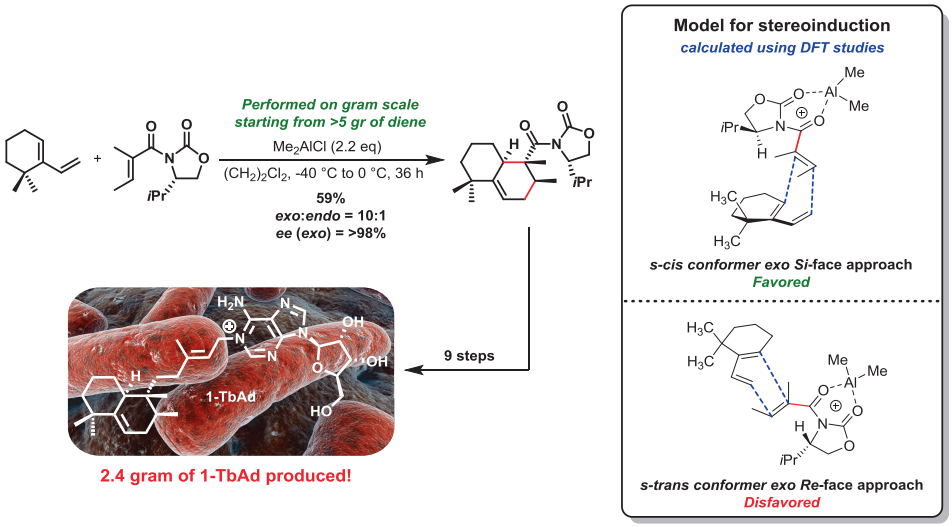
Scheme 2. *The discovery and biosynthesis of 1-tuberculosinyl adenosine, and is development into a chemical marker for tuberculosis.*

Further analysis showed that 1-TbAd is not only a highly abundant, but also a *Mycobacterium tuberculosis*-specific molecule. This led to the hypothesis that 1-TbAd can act as a specific chemical marker and be used to diagnose tuberculosis. Its potential as a chemical marker was shown by reversed phase HPLC-MS analysis of whole lung homogenates of six tuberculosis infected BLB/C mice. In all samples 1-TbAd was readily detected.

From the chemical marker studies, an isomer of 1-TbAd was detected as well. Again, mass spectrometry and NMR spectroscopy were used to elucidate the structure which was found to correspond to 1-TbAd's pseudo-isomer N^6-TbAd. A chemical synthesis

reinforced this conclusion and also showed the conversion of 1-TbAd into N^6-TbAd to proceed via a Dimroth rearrangement.
The identification of these novel *Mycobacterium tuberculosis*-specific molecules, in particular that of 1-TbAd, is of significant importance. This molecule has shown to be usable as a chemical marker and is produced by the virulence associated enzyme Rv3378c. Although the role this molecule plays in the virulence/survival of *Mycobacterium tuberculosis* in macrophages remains unknown, we believe an important step has been made to unravel this complex mechanism.

With the discovery of 1-TbAd there is a demand for enantiopure reference material. Unfortunately, the isolation of 1-TbAd from its natural source is laborious and provides only very small quantities. This problem has been solved by the stereoselective synthesis of the natural product as shown in chapter 4 (Scheme 3).
Our venture into the 1-TbAd total synthesis started with a chiral pool strategy. Naturally occurring sclareolide was chosen as a starting material but the 1-TbAd synthesis ground to a halt as our envisioned route failed. A detour was also investigated but unfortunately to no avail.

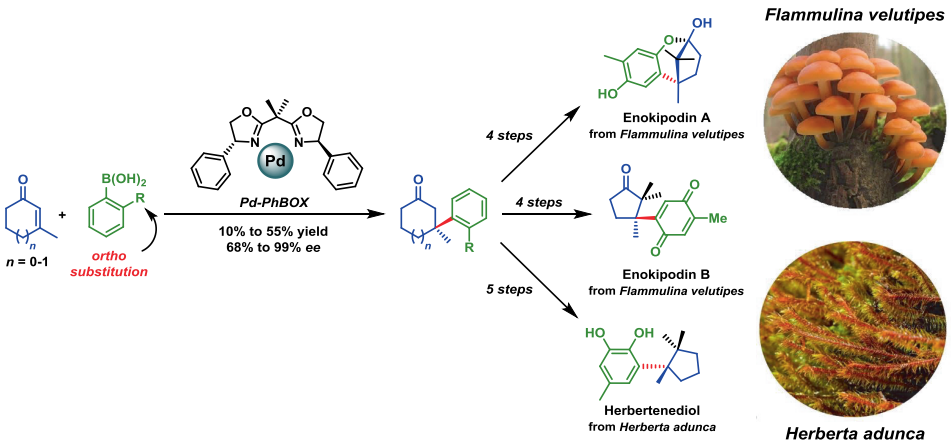


Scheme 3. *A highly stereoselective synthesis of the halimane skeleton in the gram-scale total synthesis of 1-TbAd*

The second strategy chosen to construct the halimane core-structure of 1-TbAd involved a Diels-Alder reaction. A plethora of catalyzed Diels-Alder cycloadditions, ranging from DNA catalysis, organocatalysis to transition metal catalysis, were performed but the Diels-Alder adduct could not be produced at all or with moderate diastereo- and enantioselectivities.

After this investigation we turned our attention to the use of chiral auxiliaries as part of the dienophile. The use of the Evans chiral oxazolidinone proved to be fruitful as the Diels-Alder adduct was produced with high diastereoselectivity (*exo* : *endo* = ~10:1) and excellent enantioselectivity (>98% *ee*) for the desired *exo* diastereomer. Completing the synthesis of enantiopure 1-TbAd took an additional nine steps and produced ~2.5 gram of natural product, sufficient for biological studies. Besides the first asymmetric total synthesis of 1-TbAd, congeners of the natural product (2'-deoxy 1-TbAd, (*Z*)-1-TbAd, and $^{13}C_{ribose}$-labelled 1-TbAd) and N^6-TbAd were also constructed.
The chapter ends with an investigation into the mechanistic course of the Diels-Alder reaction. A model to explain the stereoselectivity was proposed and validated by *in silico* studies. It was found that the *s*-cis conformer of the dienophile dictates the reaction which proceeds following the Curtin-Hammet principle.

After the synthesis of *Mycobacterium tuberculosis* isolates, the focus was shifted to palladium-catalyzed asymmetric conjugate additions in the construction of several terpene-based natural products. Chapter 5 describes the development of methodology to construct sterically congested benzylic quaternary stereocenters bearing *ortho*-substituents (Scheme 4).

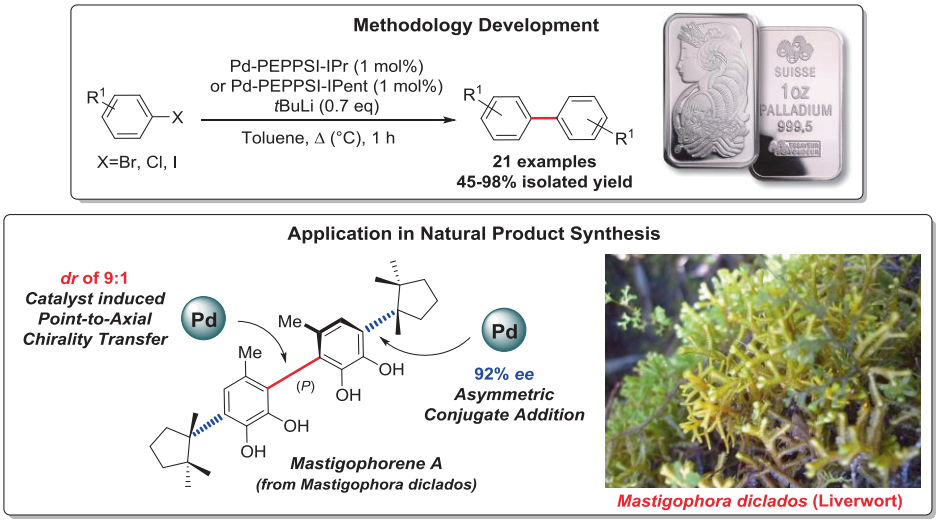


Scheme 4. *Development of the Pd-catalyzed asymmetric conjugate addition of ortho-substituted arylboronic acids to 3-methyl substituted cyclic enones, and its application in natural product synthesis.*

Although recent advances in the palladium-catalyzed Michael addition of arylboronic acids to β-substituted enones have been made, reactions with *ortho*-substituted arylboronic acids were shown to be problematic. Optimization of previous in-house developed methodology was performed resulting in the successful addition of *ortho*-substituted arylboronic acids with generally good enantioselectivities. Unfortunately the isolated yields were moderate at best which was attributed to significant protodeboronation of the arylboronic acid. Despite this feature the reaction was shown

to be applicable in the shortest asymmetric total synthesis of herbertenediol and enokipodin A and B.

The short asymmetric total synthesis of herbertenediol set the stage for a concise stereoselective synthesis of its dehydrodimer mastigophorene A, as presented in chapter 6. The biaryl axis of mastigophorene A and B is chiral and is a challenging functionality to install stereoselectively (Scheme 5).

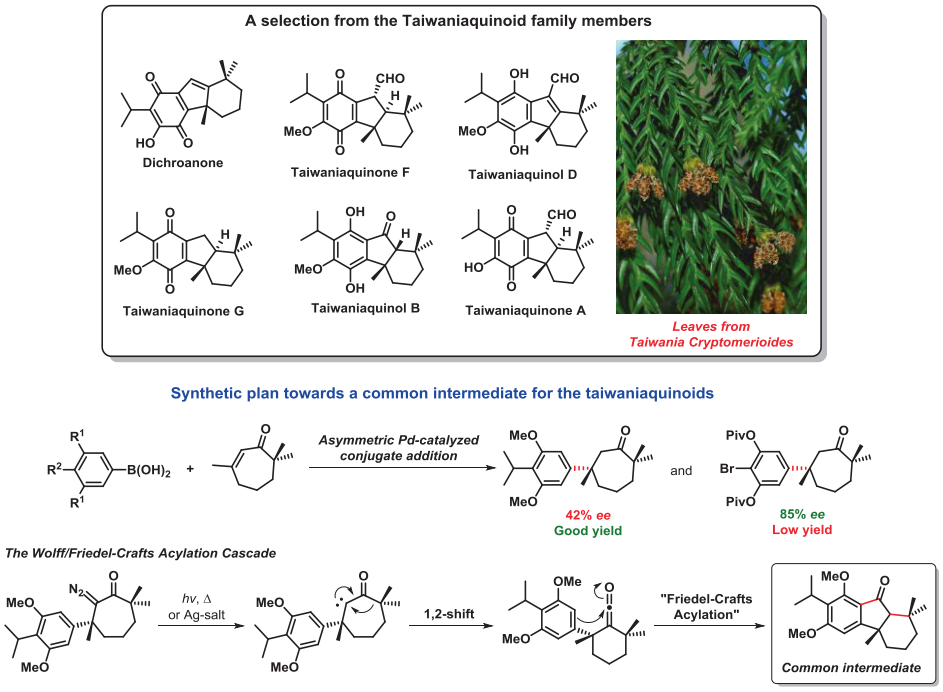


Scheme 5. *Development of the Pd-catalyzed homo-coupling of aryllithium reagents and its application in the shortest atropselective synthesis of mastigophorene A.*

Over the past three years, the Feringa laboratory made significant advances in the palladium-catalyzed cross-coupling of organolithium reagents. Concurrent with our work on herbertenediol, the Feringa group developed methodology for the hetero- and homo-coupling of aryllithium reagents with aryl halides. Combining our efforts led to the shortest asymmetric total synthesis of mastigophorene A in only eight steps. Surprisingly, the chiral biaryl axis was installed with high diastereoselectivity, induced by the seemingly remote benzylic quaternary stereocenter on the *para*-position via so-called catalyst induced point-to-axial chirality transfer.
The combination of the described asymmetric conjugate addition and the in this chapter presented homo-coupling of aryllithium reagents, were responsible for the considerable improvement over the 20+ steps needed in previous atropselective total syntheses of mastigophorene A.

In the final chapter of this dissertation, chapter 7, an investigation into the total synthesis of the taiwaniaquinoid family is presented. Bearing an unusual [6,5,6]-*abeoabietane* skeleton, together with a benzylic quaternary stereocenter, this class of molecules opposes a significant synthetic challenge.

Our synthetic route was based on the recently developed Pd-catalyzed asymmetric conjugate additions of arylboronic acids to cyclic enones. A novel feature of this plan was the addition to a rarely used substituted cycloheptenone. The construction of the [6,5,6]-*abeoabietane* core structure was envisioned to be feasible by means of a ring contraction/acylation type domino reaction (Scheme 6).



Scheme 6. *Overview of the general synthetic plan explored towards an asymmetric total synthesis of several taiwaniaquinoid family members.*

A considerable number of reactions was performed to achieve the asymmetric synthesis of the [6,5,6]-*abeoabietane* skeleton. However, the conjugate addition proved to be very challenging although eventually we managed to achieve an enantioselectivity of 85% *ee*. The isolated yield for the Michael adduct proved to be low and therefore the route was continued with racemic material. Several ring contraction strategies were investigated however to no avail.

Although the synthesis of the [6,5,6]-*abeoabietane* core was not achieved, the synthetic plan should not be considered a dead end. Several other ideas for ring contraction strategies are presented in chapter 7 and work will continue to complete the total synthesis of the taiwaniaquinoids.